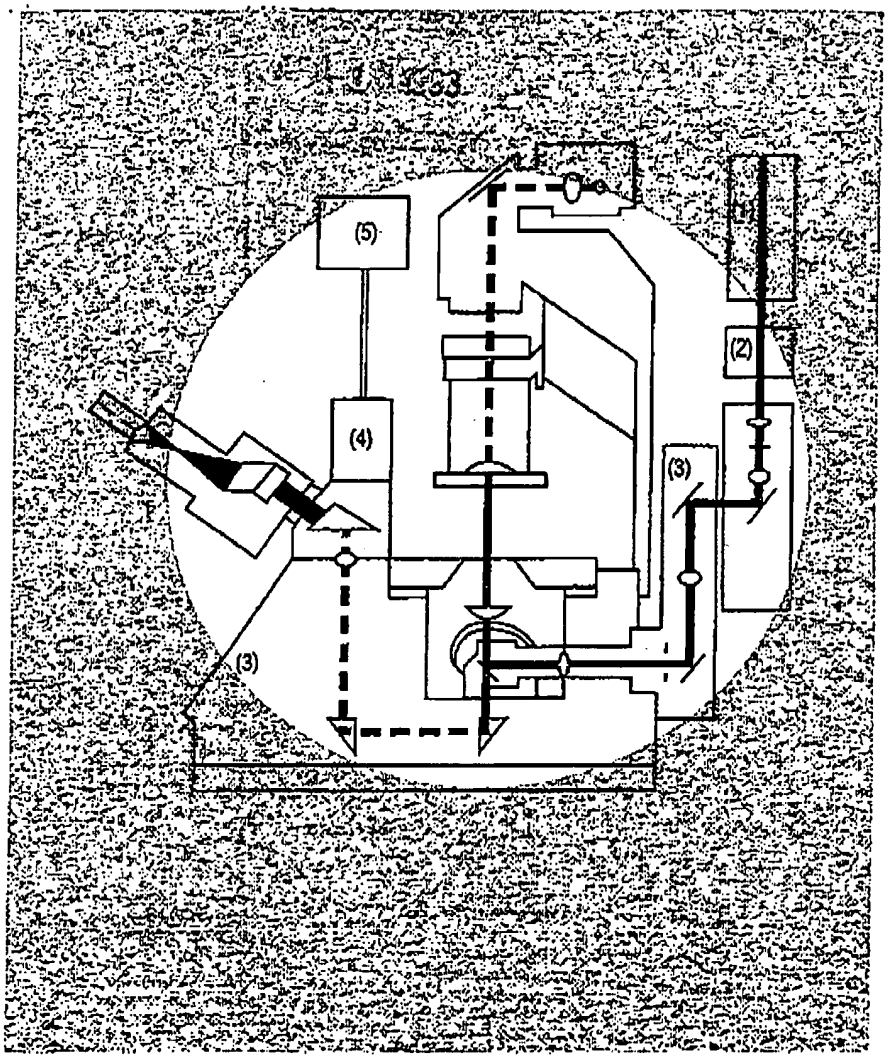
EXHIBIT 7

23 · 1 · 93
January–February 1993
(Released March 1993)

International Journal on Haemostasis and Thrombosis Research

# Haemostasis




S. Karger
Medical and Scientific
Publishers
Basel · Freiburg
Paris · London
New York · New Delhi

KARGER

Original Paper

Haemostasis 1993;23:8–12

# Effects of Aspirin on Embolization in an Arterial Model of Laser-Induced Thrombus Formation

*M.H. Vesvres*
*F. Doutremepuich*
*M.Cl. Lalanne*
*Ch. Doutremepuich*

Laboratoire d'Hématologie
CJF INSERM 88/13, Bordeaux,
France

**Key Words**
Arterial thrombosis
Laser
Aspirin
Embolization

**Abstract**
This model of arterial thrombosis induced by laser was used to evaluate the effect of aspirin (Aspegic®) on embolization. A partial occlusion was induced in small mesenteric arterioles (diameter 35–40 μm) with an Argon Laser. The laser induced the formation of a vessel wall lesion with damage of endothelial cells. Thrombus formed within seconds after the laser lesion and grew rapidly. Embolization began within the minute following the laser flash. Thrombus formation and embolization were repetitive phenomena. The duration of embolization was 6.50 ± 0.84 min in the control group. Then the thrombus became stable and partially obstructed the vessel lumen. The administration of aspirin at three doses (50, 100, 200 mg/kg) by intramuscular injection, 15 min before the laser injury, induced three different phenomena: (1) an increase of the number of laser injuries required for the thrombus formation; (2) a dose-dependent decrease in the duration of embolization, and (3) a dose-dependent decrease in the number of emboli. The highest dose injected induced the strongest reduction in the duration of embolization and the number of emboli.

## Introduction

Arterial thrombosis develops at endothelial lesions. In response to damage of the vessel wall, platelets adhere rapidly to the collagen, a component of vascular subendothelium. Afterwards they release the contents of their granules and aggregate reversibly [1, 2]. The aggregates can be swept away by the blood flow and a new thrombus forms again.

Received:
January 20, 1992
Accepted in revised form:
March 8, 1992

C. Doutremepuich
Laboratoires d'Hématologie
CJF INSERM 88/13
3, place de la Victoire
F-33076 Bordeaux (France)


0301–0147/93/
0231–0008$2.75/0

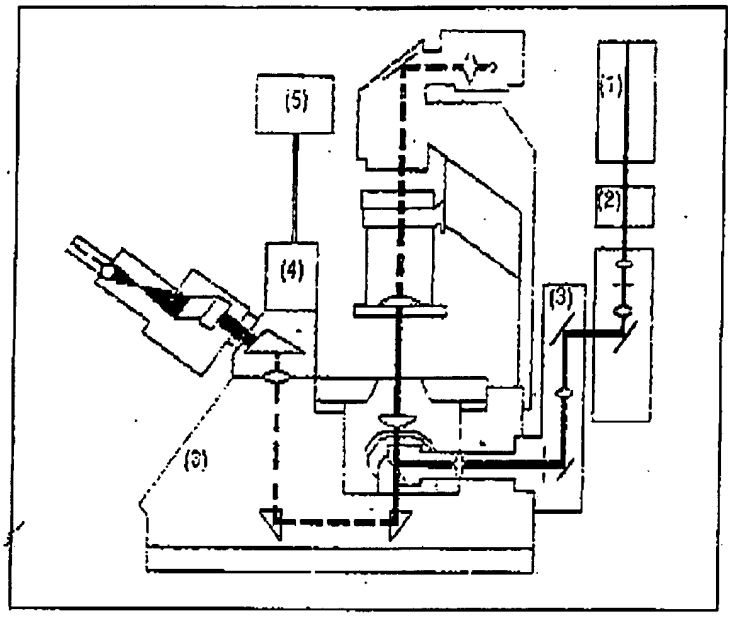



**Fig. 1.** Schema of the laser combination with the microscope. 1 – Argon laser; 2 – obturator; 3 – inverted microscope axiovert 10; 4 – camera DXC 107 P; 5 – monitor PVM 1440 QM. The path of the laser beam was in a continuous line and the path of the light was in a dotted line.

Such aggregates in humans can be responsible for cerebral transient ischemic attack and minor ischemic stroke.

Different models of arterial thrombosis have been developed in animals. Arterial thrombosis induced by laser beam has the greatest resemblance the pathological processes resulting from a lesion observed in human disorders. Indeed, with this model, it was possible to obtain a restricted destruction of the endothelial wall. The endothelial injury was induced by an argon laser in rat mesenteric arterioles with a diameter of 35–40 μm [5, 6].

The aim of this study was to induce a vessel wall lesion and to evaluate the effect of aspirin (Aspegic®) on thrombus embolization. Different evaluation criteria on the drug effects were studied. The number of laser lesions required to induce thrombus formation was determined in control animals and in rats which had received the drug. Other parameters studied were the number of emboli and the duration of embolization.

## Material and Methods

*Animals*

The investigations were carried out on male Wistar rats from the Depre breeding center (Saint-Doulchard, France), weighing 430–450 g. A stabling period of 8 days preceded the beginning of experimentation. The rats were anesthetized by intramuscular injection (250 mg/kg BW) of thiopental sodium (Nesdonal®), Laboratoire Specia, France). After a median laparotomy, an intestinal loop was mounted on the microscope table. Arterioles with a diameter of 35–40 μm, were isolated. Two arterioles were investigated per rat. Five rats were studied in each group.

*Material*

Vascular lesions were induced by an argon laser (Stabilite 2016, Spectra-Physics, France). The wavelength used was 514.5 nm. The energy was adjusted to 80 mW. The laser beam was applied to the arteriole for 1/30 s. The laser was mounted on an inverted microscope (Axiovert 10, Zeiss, France). The observations were achieved through a ×100 oil-immersion lens. A video camera (colour camera CCD) was attached to the microscope and was connected to a monitor through which thrombous and embolus formation could be visualized (fig. 1).

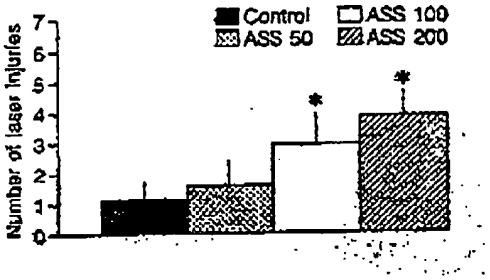



**Fig. 2.** Number of laser injuries required to induce thrombus formation. ASS = Aspegic. The doses are expressed in mg/kg. Laser energy: 80 mW. Exposure time: 1/30 s. The data are presented as means ± 1 SD. In each group of 5 rats, ten vessels are investigated. * $p < 0.05$ – Significant changes compared to controls.

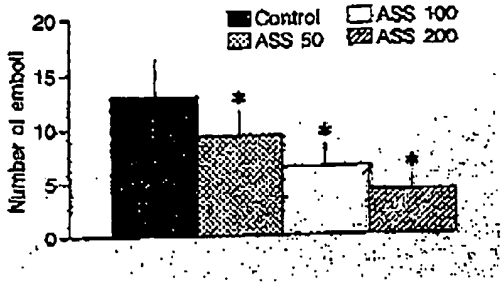



**Fig. 3.** Number of emboli mobilized after laser injuries. ASS = Aspegic. The doses are expressed in mg/kg. Laser energy: 80 mW. Exposure time: 1/30 s. The data are presented as means ± 1 SD. In each group of 5 rats, ten vessels are investigated. * $p < 0.05$ – Significant changes compared to controls.

*Parameters Studied*

Three parameters were assessed:

(1) The number of laser injuries required to induced thrombus formation. If no thrombus formed after the first laser application, another flash laser was applied to the vessel one minute after the first lesion. Laser application was stopped when adhesion and platelet aggregation began and when the thrombus was visible.

(2) The number of emboli which were removed by the bloodstream after laser injuries.

(3) The duration of embolization.

*Drug Tested*

The aspirin preparation used (Aspegic) is an acetylate of lysine (Laboratoire Synthelabo, France). Three doses were tested: 50, 100 and 200 mg/kg. Fifteen minutes before the experiments had begun, rats were treated with one dose of Aspegic by intramuscular injection. Five rats and two vessels in each rat were investigated per group.

*Statistics*

Statistical analyses are carried out on PCSM® Software (Deltasoft, France). Results are expressed as mean ± 1 SD. The data were evaluated using nonparametric tests: Kruskal-Wallis test and Mann-Whitney test between the group of animals which had received Aspegic and the placebo group. p values less than 0.05 were regarded as significant.

## Results

The results obtained with control rats and after treatment with aspirin (Aspegic) are summarized in figures 2–4.

In control animals, 1.20 ± 0.42 laser injuries were necessary to induce the formation of a platelet thrombus (fig. 2). Injection of Aspegic led to an increase of the number of the laser injuries whatever doses were administered. Moreover, the administration of 50 mg/kg of Aspegic induced a slight but no significant increase of this number. This number of laser injuries was only significantly increased after the administration of 100 and 200 mg/kg.

The second parameter studied was the number of emboli which were released. The administration of Aspegic whatever the injected doses (50, 100 and 200 mg/kg) significantly decreased embolus formation (fig. 3). The percent decrease in the number of emboli was 28, 50 and 68% after the administration of 50, 100 and 200 mg/kg of Aspegic, respectively.

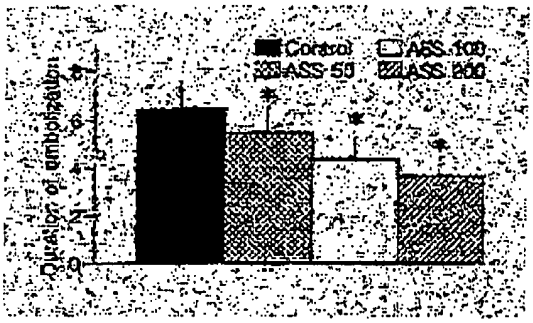



**Fig. 4.** Duration of embolization. ASS – Aspegic. The doses are expressed in mg/kg. Laser energy: 80 mW. Exposure time: 1/30 s. The data expressed in minutes, are presented as means ± 1 SD. In each group of 5 rats, ten vessels are investigated. * $p < 0.05$ – Significant changes compared to controls.

The duration of embolization was determined in control rats and in rats which had received Aspegic. In control rats, embolization persisted for 6.50 ± 0.84 min. Aspegic administration induced a dose-dependent decrease in the duration of embolization (fig. 4). The percent of decrease of this parameter was 15, 32 and 41% after injection of 50, 100 and 200 mg/kg of Aspegic, respectively.

These results show that Aspegic was effective in the prevention of embolization. The injection of the highest dose (200 mg/kg) induced the strongest effect on the number of emboli and the duration of embolization.

## Discussion

Argon laser damages the vessel wall including endothelial cells. The injury was restricted to a few cells. In less than 1 min, the lesion leads to adhesion and reversible platelet aggregation. The growing thrombus embolizes and then it forms again until it becomes stable.

In control rats, a mean of 1.20 ± 0.42 laser injuries were required to induce thrombus formation while the administration of Aspegic significantly increased this number. Aspirin is known for its antithrombotic effects [6, 7]. Aspirin inhibits platelet aggregation induced by arachidonic acid, collagen, and the second aggregation induced by ADP, epinephrin, thrombin. It is an irreversible inhibitor of platelet cyclooxygenase [8, 9].

After injection of 50 mg/kg of Aspegic, a mean of 1.60 ± 0.51 laser injuries were required for thrombus formation. As Aspegic inhibits platelet aggregation, it is possible that a more important lesion must be induced by laser to result in the development of a thrombus in the vessel lumen.

Indeed, the pro-aggregatory activity of the different layers constituting the vessel wall increased from the internal elastic lamina to the adventitia [10]. A more extensive lesion in the vessel wall which could reach the media, could explain thrombus formation in spite of the presence of Aspegic in the circulation. The higher injected dose, the greater number of laser injuries required for thrombus formation compared with the control, must be important so the lesion must be more extensive.

The second parameter studied is the number of emboli which are removed by the blood-stream after laser injuries. Thrombus embolization differed significantly from that observed in control rats. The number of emboli decreased with increasing dose. Different hypotheses can be put forward. It is most likely that the thrombus formed is smaller than in control rats and the final structure of the thrombus is produced more rapidly, reducing the time period during which the embolization can occur. This fact is supported by the analysis of the duration of embolization. Indeed, the administration of Aspegic induces a significant decrease of this duration whatever the injected doses.

This study, two effects of Aspegic have been demonstrated: it induces a diminution in the number of emboli and it reduces the duration of embolization. The stronger effect is obtained after the injection of the higher doses tested (50, 100 and 200 mg/kg).

In spite of the results of a study from 'the dutch TIA trial study group' [11], in which the authors found that 30 mg of aspirin daily had similar effects than the dose of 283 mg in the prevention of vascular events in patients with a transient ischemic attack or minor stroke, our study indicates that the injection of high doses of Aspegic is more effective against thrombus formation and the embolization at least in rats.

## Conclusion

Aspegic is an antiaggregant drug which inhibits platelet cyclooxygenase. Its use in thrombosis prophylasis is broad (ischemic stroke, prevention of myocardial infarction and cerebrovascular diseases).

This study shows results of Aspegic injections at different doses (50, 100 and 200 mg/kg) on the development of the thrombus. The effect of Aspegic on the number of emboli and on the duration of the embolization is stronger if higher doses are injected.

Aspegic in this study inhibited thrombus formation, the number of emboli formed and the duration of embolization. These effects were clearly dose dependent.

## References


1 Born G, Görög P, Kratzer M: Aggregation of platelets in damaged vessels. Proc R Soc Lond [B] 1981;294:241–250.

2 Tschopp TB, Baumgartner H, Silberbauer K, Sinzinger H: Platelet adhesion and platelet thrombus formation on subendothelium of human arteries and venous exposed to flowing blood in vitro: A comparison with rabbit aorta. Haemostasis 1979;8:19–29.

3 Fuster V, Chesebro J: Antithrombotic therapy: Role of platelet inhibitor drugs. Management of arterial thromboembolic and atherosclerotic disease. Mayo Clin Proc 1981;56:265–273.

4 Woimant F, Lecoz P, Haguenau M, Pepin B: Le risque hémorragique d'un accident ischémique cérébral récent sous traitment anticoagulant. Semaine Hôp Paris 1986;3033–3034.

5 Weichert W, Breddin HK, Staubesand J: Application of a laser induced endothelial injury model in the screening of antithrombotic drugs. Semin Thromb Haemost 1988;14:106–114.

6 Weichert W, Pauliks V, Breddin HK: Laser induced thrombi in rat mesenteric vessels and antithrombotic drugs. Haemostasis 1983;13:61–71.

7 Weichert W, Breddin HK: Antithrombotic effects of acetylsalicylic acid (ASA) and pentoxifylline in laser induced thrombosis in rat mesenteric vessels. Vasa Band 1985;14:Heft 3.

8 Buchanan M, Butt R, Markmann B, Hirsh J: Effects of aspirin and salicylate on platelet function. Thromb Haemost 1983;50:101.

9 Harker L, Fuster V: Pharmacology of platelet inhibitors. J Am Coll Cardiol 1986;8:21B–32B.

10 Moncada S, Herman AG, O'Higgs E, Vane J: Differential formation of prostacyclin (PGX or PGI2) by layers of the arterial wall. An explanation for the antithrombotic properties of vascular endothelium. Thromb Res 1977;11:323–344.

11 The Dutch TIA Trial Study Group: A comparison of two doses of aspirin (30 mg vs. 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke. N Engl J Med 1991;325:1261–1266.